Reproductive Pattern of Sea Cucumber, Holothuria scabra at Two Different Sites in Sabah, Malaysia

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Abstract
Holothuria scabra is one of valuable sea cucumber in Sabah as it can give a high quality of *beche-de-mer* that can be source of income for the fisherman. High demand of this species has led to overexploitation and overfishing, thus production in hatchery is crucial to overcome this problem since this species able to be reared in captivity. The presence study was conducted for 14 months started from July 2015 until August 2016 at two places Kudat (N06°49'24.4", E116°51'42.0") and Kunak (N04°39'52.05", E118°15'49.01") Sabah, Malaysia. Gonad index (GI) and microscopic examinations were used to evaluate monthly variations of gonad maturation. Annual reproductive pattern was observed at Kudat where the highest peak of GI was in July 2015 (1.678 ± 1.079%) and the lowest in July 2016 (0.00 ± 0.00%) whereas in Kunak continuous pattern recorded with the highest peak in September 2015 (3.491 ± 1.699%) and the lowest GI in Feb 2016 (0.184 ± 0.097%). Size at first sexual maturity in Kudat and Kunak were approximately 99 g and 101 g, respectively and in length approximately 174 mm for both places.
Keywords: Holothuria scabra, reproductive pattern, gonad index, Kudat, Kunak

1. Introduction

Sea cucumber, members of Class Holothuroidea in phylum Echinoderm and also referred as holothurians or holothuroids (Preston, 1993; Hamel *et al.,* 2001; Sicuro and Levine, 2011; Kuganathan, 2014; Kamarudin, Rehan, Hashim, & Usup, 2010) is a sessile marine invertebrates found at the benthic areas (Rahman, 2014). Aspidochirote holothuroid, *Holothuria scabra* or commonly name as sandfish (Plotieau, Baele, Vaucher, Hasler, Koudad, & Eeckhaut, 2013; Ramofafia, Byrne, & Battaglene, 2003; Rasolofonirina, Vaitilingon, Eeckhaut, & Jangoux, 2005) has elongated body shape similar to cucumber (Pangkey, Lantu, Manuand, & Mokolensang, 2012) with a hollow body that is often covered by fine sand (Kuganathan, 2014; Purcell, Samyn, & Conand, 2012). *H. scabra* can be found in sandy environment throughout the tropical Indo-Pacific (Mercier, Battaglene, & Hamel, 2000; Pangkey *et al.,* 2012; Agudo, 2006) at depths of 2-25 m or more (Pitt and Duy, 2003).

Most holothurian are broadcast spawners, releasing their sperm and oocytes to the water column (Purcell, Lovatelli, Vasconcellos, & Ye, 2010; Kuganathan, 2014) and it can be reproduced by sexual and asexual reproduction (Preston, 1993; Hoareau and Conand, 2001). Yet, in *H. scabra*, only sexual reproduction occurred (Purcell *et al.,* 2010; Pangkey *et al.,* 2012; Kuganathan, 2014; James, 1989).

The reproductive cycle of this species has been studied at most of its geographical range, from Red Sea to the Philippines and to New Caledonia (Rasolofonirina *et al.,* 2005). Behavior of sandfish generally illustrate that spawning is likely to occur at any times (Agudo, 2006). Most of the studies show that *H. scabra* has different peak of spawning in different parts of the world (Pangkey *et al.,* 2012). However, according to Ramofafia *et al.,* (2003) *H. scabra* show two main basic reproductive patterns: seasonally predictable spawning at high latitudes and aseasonal spawning at low latitudes (Ramofafia *et al.,* 2003).

Holothuria scabra is the only species that currently produced in mass production (Choo, 2008; Hasan, 2005) and India is the first country that been succeeds to culture *H. scabra* in hatchery. It is followed by the other countries such as Australia, Indonesia, Maldives and Solomon Islands where the procedures for mass culture of *H. scabra* are well established and practiced (Rahman, 2014; Choo, 2008). The production from the hatchery and aquaculture are aiming to restore the depleted population in the wild (Hamel, Conand, Pawson, & Mercier, 2001). Besides, it is also aimed to support the continuing demand of this organism in aquaculture and biomedical research programs (Rahman, 2014).

Semporna, Sandakan, Kudat, Kota Marudu, Kota Belud and Kota Kinabalu are the landing ports of sea cucumber in Sabah (Choo, 2008). Nevertheless, from mid-1990s, Kota Marudu and Kota Belud have no landings report indicate the populations in those areas have been depleted (Choo, 2008). In the presence study, Kudat and Kunak have been selected as the availability of *H. scabra* can support number of samples required along the sampling period.

Decrease in number of landings reported from previous studies showed that study on reproductive pattern of this species is important for broodstock management in order to increase its seed production in captivity and also to restock the population in the wild that been overexploited. Therefore, this study was conducted to get more information on reproductive biology of *H. scabra* in Kudat and Kunak.

2. Materials and Methods

Two sampling sites were selected based on the availability of the *H. scabra* which were; Limau-Limauan Kudat (N06°49'24.4", E116°51'42.0") at west coast and Telaga Tujuh Kunak (N04°39'52.05", E118°15'49.01") at east coast of Sabah (Figure 1). Sampling was conducted on monthly for each place starting from July 2015 until August 2016 (14 months). On each sampling occasion, approximately 15 samples were collected randomly. Uniform size of samples was collected to avoid any bias in studying the reproduction pattern of *H. scabra* (Hoareau and Conand, 2001).
Figure 1 near here

2.1 Gonad Index (GI)

The samples were weighted to the nearest 0.01g by using analytical weight balance after left in a dry container for five to ten minutes to expel the water from the body. The total length of the samples were measured by using Vernier caliper. Each sample was dissected at the ventral part to remove the gonad and the germinal tubule. Then, the gonad was weighted to the nearest 0.01g. After that, the gutted body weight of the sample was weighted again. The gonad index was calculated by using the following equation:

$$GI\ (\%) = GW/GBW \times 100$$

Where:

GW = Gonad weight (g)

GBW = Gutted body weight (g)

The percentage (%) of male and female at Kudat and Kunak for each month were calculated by using equation below:

$$\text{Percentage of male or female (\%)} = (n_{m/f})/N \times 100$$

Where:

n_m = Number of male

n_f = Number of female

N = Total number of individuals for each month

2.2 Identification of maturity stages in H. scabra

Maturity stages in *H. scabra* was identified by using microscopic observation of a fragment of the gonad (Demeuldre and Eeckhaut, 2012).

Histological examination was performed for the microscopic assessment. The gonad of *H. scabra* 3-5 cm long was fixed in Bouin's solution for 24 hours. Then, the gonad was stored in 70% ethanol for longer preservation. The gonad that observed under the microscope was dehydrated in graded alcohol baths (80%, 90%, 95% and 100% ethanol) and xylene. After dehydrated, the sample of gonad was embedded in paraffin and was sectioned (6 µm thick). Then, the sample was stained with haemotoxylin and eosin. Determining of gametogenetic stage relates to the staining response (Keshavarz, Mohammadikia, Dabbagh, & Kamrani, 2015) and it was defined into five gametogenic stages as shown in Table 1.
Table 1 near here

2.3 Size and weight at first maturity

Size at first maturity of population was defined as the total length (TL_{50}) or gutted body weight (GBW_{50}) at which gonads of 50% of the individuals were mature. It was determined by plotting the percentage of individuals with mature gonads against classes of gutted body weight or length (Drumm and Loneragan, 2010; Conand, 1993).

2.4 Maturity Stage Percentage

The percentage of the individuals based on developmental stages at each sampling was calculated by using the following equation:

$$\text{Percentage of individuals based on developmental stages (\%)} = n/N \times 100$$

Where:

n = Number of individuals in developmental stage

N = Total number of individuals for each month

2.5 Statistical analysis

The size distribution of sample between Kudat and Kunak was analysed by using independent T-Test. Non parametric Chi-square test was used to check on the significant of the sex in each place. Normality distribution of GI was checked by using Shapiro-Wilk W test; Kruskal-Wallis test was then used to check on differences of gonad indices between months.

3. Results

3.1 Size distribution

The size distribution of sampled *H. scabra* showed in Table 2. There was significant difference ($p<0.05$) between total length, total body weight and gutted body weight between Kudat and Kunak but no significant difference ($p>0.05$) of gonad weight between these two sites.

Table 2 near here

3.2 Sex percentage

A total of 210 *H. scabra* were collected from Kudat where 16 samples (7.6%) were females and 14 samples (6.7%) were males while the rest, 180 samples (85.7%) were undetermined sex. The undetermined sex samples in Kudat either has no gonad or undeveloped gonad which was unable to be identified. The sex ratio female to male is 1.14: 1 and Chi-square test showed slight dominance of female was not significantly different ($x= 0.13$; $df= 1$; $p> 0.05$).

Both male and female in Kudat can be found in November 2015, February 2016, March 2016, May 2016 and August 2016 (Table 3). The rest of the months, only one sex (6.7%) found either male or female. In July 2016, no male or female found since no *H. scabra* was with gonad during that month.

Table 3 near here

In Kunak, 159 samples were dissected whereby 43 males (27.0%) and 29 females (18.2%) were found and give ratio 1.48: 1 of male to female. The Chi-square test indicated the sex-ratio was not significantly different from 1:1 ($x=2.72$; $df= 1$; $p> 0.05$).

Both male and female of *H. scabra* can be found in all sampling occasions in Kunak except during August 2015 where only female was found (Table 4).

Table 4 near here

3.3 Gonad index

Based on 14 months sampling period, Kudat shows annual pattern of reproductive cycle while Kunak has continuous pattern (Figure 2). The highest GI in Kudat was recorded in July 2015 (1.678 ± 1.079%) and the lowest GI value was in July 2016 (0.00 ± 0.00%). In Kunak, the highest GI was recorded in September 2015 (3.491 ± 1.699%). The lowest GI was recorded in February 2016 (0.184 ± 0.097%).

GI in Kudat and Kunak were not normally distributed. Comparison of GI starting from July 2015 until August 2016 showed no significant ($p>0.05$) difference among those months in Kudat and Kunak.

Figure 2 near here

3.4 Histological examination

3.4.1 Spermatogenesis

Testes development in *H. scabra* divided into five stages (Table 5). Spent stage (Stage I) described as the tubule lumen has empty space. A few spawned spermatozoa were seen. Recovery (Stage II) showing a thin layer of germinal cell at the periphery of the tubule and the lumen usually empty. Then, the testes develop to the growing stage (Stage III) which was described as highly convoluted tubule wall with spematogonia along the germinal epithelium. Maturation (Stage IV) showing numerous spermatozoa found in the lumen. Spawning (Stage V) showed tubule wall very thin and mature testes packed with spermatozoa.

Oogenesis

Gametogenesis stage in female of *H. scabra* was divided into five stages (Table 5). Ovary in Stage I was wrinkled and shrunken with a few scattered oogonia can be seen. Recovery stage (Stage II) where small pre-vitellogenetic oocytes in diameter may present and thickening of tubule wall. Growing stage (Stage III) showing oocytes starts to grow and the size varied in diameter. Ovary in maturation stage (Stage IV) has large oocytes filled almost completely the lumen. The tubule wall is thinner and increased in diameter. Spawning (Stage V) described as lumen packed with large, rounded oocytes.

Table 5 near here

3.5 Size and weight at first maturity

There is a specific minimal body size for an individual becomes sexually mature (Omar, Abdel Razek, Abdel Rahman, & El Shimy, 2013). In this study, gutted body weight and length were used to determine the first sexual maturity size. The size at first sexual maturity of *H. scabra* based on gutted body weight (GBW_{50}) in Kudat and Kunak were approximately 99 g and 101 g (Figure 3a), respectively and in total length (TL_{50}) approximately 174 mm for both places (Figure 3b).

Figure 3a and 3b near here

3.6 Maturity stages

In Kudat, GI is quite high in the first five months started from July until November 2015 (Figure 4). Mean GI can be related to the maturity stage of *H. scabra* during that month. From July to November 2015, most sample were in Stage 4 and 5. After November 2015, a tremendous decreasing of GI in December 2015 and continuously until April 2016. During this period, samples of *H. scabra* were in Stage 1 to Stage 4. A relatively small peak occurred in May 2016 since some of samples were in Stage 5. After May 2016, GI decrease continuously until July 2016. In July 2016 no sample with gonad found, thus lowest GI recorded was recorded (0.00 ± 0.00%) In August 2016, high GI was recorded as most of the sample were in Stage 5. Three high peaks of GI in Kudat were recorded during July 2015 (1.678 ± 1.079%), November 2015 (1.578 ± 1.202%) and August 2016 (1.668 ± 0.752%).

Figure 4 near here

In Kunak, gametogenesis appeared continuous with periods of enhanced activity. Mature stage (stage 4 and 5) was observed in most of sampling occasions (Figure 5). The highest peak was recorded in Sep 2015 (3.491 ± 1.699%) as gonads were in stage 4 and 5. GI drop gradually in Oct and Nov 2015, 0.498 ± 0.229% and 0.254 ± 0.145%, respectively as gonad was in growing stage (stage 3). The lowest GI was recorded in Feb 2016 (0.184 ± 0.097%) because some of the gonad had released the gametes (spent). Constant increase of GI on Mac to Apr 2016 and then drop a bit in May and July 2016. The GI rise drastically in Aug 2016 (1.794 ± 0.955%) as the gonad developed to be matured.

Figure 5 near here

3.7 Relationship between Gutted body weight with gonad index and gonad weight

Relationship between gutted body weight with gonad index and gonad weight were shown in Figure 6a and 6b. Data in both figures consist of all samples from Kudat and Kunak. Pearson correlation show no significant ($p>0.05$) correlation between gutted body weight with gonad index and gonad weight. The gonad index and gonad weight were varied across the gutted body weight of the samples.

Figure 6a and 6b near here

4. Discussion

Holothuria scabra is a gonochoric species but cannot be distinguished externally (Pangkey *et al.,* 2012; Kuganthan, 2014). The sex can only be determined by observing the spawning behavior and through macroscopic and microscopic examination (Agudo, 2006). The sex ratio of population at both sites, Kudat and Kunak was similar in many holothurians specifically for *Holothuria* species (Kazanidis *et al.,* 2014; Mezali *et al.,* 2014; Navarro *et al.,* 2012; Asha and Muthiah, 2008; Rasolofonirina *et al.,* 2005; Guzman *et al.,* 2003; Ramofafia *et al.,* 2000).

Holothuria scabra in the present study had a total length range 103.66 to 278.70 mm with mean 174.85 ± 1.27 mm in Kudat and 51.28 to 261.48 mm with mean 162.66 ± 2.68 mm in Kunak. This observation shows that sample in both sites are almost the same to other observation of same species, in South-Western Indian Ocean which has size range 142.00 ± 14.00 to 238 ± 27.00 mm (Rasolofonirina *et al.,* 2005). *H. scabra* in Mahout Bay, Sultanate of Oman has a size distribution within 85 mm to 395 mm (Al-Rashdi *et al.,* 2007). *Holothuria mexicana* has a mean length of 329.30 ± 2.50 mm which is two times longer than the present study (Guzman *et al.,* 2002). Brown sandfish, *Bohadschia vitiensis* in Hurghada, Egypt had a size range 160 to 420 mm (Omar *et al.,* 2013).

In term of total body weight, *H. scabra* in Kudat had a range from 127.22 g to 453.53 g with mean 242.11 ± 3.61 g while in Kunak, ranged from 48.0 g to 529.0 g with mean 203.99 ± 7.67 g. Total body weight of *H. scabra* in both Kudat and Kunak has a slight range as compare to *H. leucopilota* in Western Indian Ocean with huge range between 81 g to 861 g (Gaudron *et al.,* 2008). Gutted body weight of *H. scabra* in Kudat ranged from 46.21 g to 187.18 g with mean 110.84 ± 1.58 g, while in Kunak ranged from 18.0 g to 249.0 g with mean 97.96 ± 3.97 g. Compared to *H. scabra* in Kenya, gutted body weight in Kudat and Kunak is two to three times smaller. In Kenya it ranged from 189.4 g to 322.0 g (Muthiga *et al.,* 2009).

Differences in size distributions may be due to the depth of sample was taken, environmental factor or the substrate type (Omar *et al.,* 2013; Kithakeni and Ndaro; 2002). There was significant difference ($p<0.05$) in total length, total body weight and gutted body weight between *H. scabra* in both sites, Kudat and Kunak. It is difficult to estimate the body dimension of sea cucumber since it has soft body and may contain highly variable water content which affects the size, shape and weight (James et al., 2015; Prescott *et al.,* 2015). However, gutted body weight was mentioned as the most consistent biometric feature to estimate the population structure (Dereli *et al.,* 2016). Gutted body weight provides weight without any water content or substrate in the body.

Size at first sexual maturity is crucial to identify the minimum size of an individual to become reproductive (Hamel and Mercier, 1996). Different authors used different parameters to determine the size of first sexual maturity. Total body weight of holothurians is difficult to be used as a parameter in order to determine the maturity since total body weight may include sediments in the digestive tracts and water in the respiratory system (Guzman *et al.,* 2003). Therefore, in this present study, total length and gutted body weight of *H. scabra* were used to determine the size at first maturity. Size at first sexual maturity in Kudat and Kunak were approximately 99 g and 101 g, respectively and their length was approximately 174 mm for both places. In terms of total length, findings in this study agreed with Kumara and Dissanayake (2015), as they described *H. scabra* in tropical region can achieve sexual maturity at size between 150 to 180 mm. *Holothuria scabra* in New Caledonia reach size at first maturity at 160 mm (Conand, 1993a) and 168 mm in Dar es Salaam (Kithakeni and Ndaro, 2002), slightly smaller than what determined in Kudat and Kunak. *Holothuria scabra* in Mauritius and India has two times bigger size compare to the present study, 210 mm and 250 mm, respectively (Purcell *et al.,* 2012b). In Abu Rhamada, *H. scabra* reported has different sexual maturity for male (160 mm) and female (185 mm) (Hasan, 2005). In Caribbean Panama, *H. mexicana* and *Isostichopus badionotus* has a minimum reproductive size between 130 mm to 200 mm (Guzman *et al.,* 2003), while *B. vitiensis* in Hurgahada area, Egypt was 245 mm for males and 261 mm for females (Omar *et al.,* 2013). Based on gutted body weight, size of sexual maturity in this study is smaller compare to *H. scabra* in New Caledonia which is 140 g (Conand, 1993a). *Holothuria sanctori* in Gran Canaria, Spain has a same size of first maturity with the presence study between 101 g and 110 g (Navarro *et al.,* 2012). Orange-footed sea cucumber, *Cucumaria frondosa* has a small size at sexual maturity which is 55 g only (Hamel and Mercier, 1996). *Actinopyga mauritiana* in Guam has a bigger size at first maturity, 158 g compare to the present study (Hopper *et al.,* 1998). Reichenbach (1999) measure the size at first maturity *H. fuscogilva* in Maldives using the total body weight which is 1500 g. This size was about six times bigger compare to total body weight of *H. scabra* in this study. Size at first maturity can be different at certain places as it is influenced by the surrounding. Kithakeni and Ndaro (2002) stated that different type of diet present at the study site may cause differences in size at first maturity. Growth of juveniles and broodstock *H. scabra* depends mainly on phytoplankton as their source of food. Another factor that may influence the differences of size at first maturity could be the depth of the sample was taken as there was a report from the divers where the large-sized of *H. scabra* usually found in deep water compared to shallow water (Kithakeni and Ndaro, 2002).

Reproductive cycle in holothurians can be annual (Chao *et al.,* 1995; Rasolofonirina *et al.,* 2005; Asha and Muthiah, 2008; Muthiga *et al.,* 2009; Benitez-Villalobos *et al.,* 2013; Omar *et al.,* 2013), biannual (Hoareau and Conand, 2001; Dissanayake and Stefansson, 2010) or even continuous pattern which can occur usually in tropical regions (Hopper *et al.,* 1998; Reichenbach, 1999; Guzman *et al.,* 2003; Ramofafia *et al.,* 2003). In this study, broodstock of *H. scabra* in Kudat has annual pattern of reproductive cycle whereas in Kunak, continuous pattern was observed. Continuous pattern in the tropical regions is not typical but annual pattern in tropical is quite unusual. Muthiga *et al.,* (2009) stated that annual reproduction usually reported at 23° N or S. This is because, at higher latitude, synchronous gametogenesis occurred with wide variations of environmental parameters (Muthiga and Kawaka, 2008; Ramofafia *et al.,* 2003). However, lack of information on seasonality experienced in the tropical area with less variation in environmental parameters reported. Some studies have emphasized on seasonally spawn of sea cucumber in tropical area (Kumara and Dissanayake, 2015). Previous study reported on *H. fuscogilva* at the tropical area also has an annual pattern reproductive cycle which is similar with finding in the present study in Kudat (Muthiga and Kawaka, 2009).

Continuous pattern of reproductive cycle in *H. scabra* in Kunak associated with the presence of mature gametes all year round. Samples in Stage 5 which is matured and ready to be spawned can be found in most months except July and October 2015 and February 2016. Other studies on the tropical holothurians noticed that mature gamete are found throughout the year (Hopper *et al.,* 1998; Ramofafia *et al.,* 2003). This continuous pattern also observed in *H. scabra* at Indonesia but different peak season with Kunak which is during September whereas, study in Indonesia conducted at three different sites; Lampung, Saugi and Ambon showed different peaks; March, April and October, respectively (Purwati, 2006). Other study on *H. scabra* in Solomon Islands shows continuous pattern with maximum gonad growth from September to December (Ramofafia *et al.,* 2003).

GI and gonad weight are variable in all body size and show no significant ($p>0.05$) correlation. This indicates that GI is not affected by the body size where small body size may have high GI and vice versa (Muthiga *et al.,* 2009; Muthiga and Kawaka, 2008). Therefore, GI is reliable indicator to predict the reproductive condition in *H. scabra* (Muthiga and Kawaka, 2008; Guzman *et al.,* 2003). There was a period which gonads were absent in the reproductive cycle as discussed by Gabr *et al.,* (2004) and Omar *et al.,* (2013) on the total resorption of tubule after spawning of gametes into the water column.

5. Conclusion

This present study gives overview on the reproductive pattern of *H. scabra* in Kudat and Kunak as well as the minimum capture size (99 g and 101 g, respectively; 174 mm) that indicate the size which is mature and have passed the reproductive period. Any size that not reach the minimum capture size should be release back into the ocean to prevent overfishing in this species. In addition, this overview may help the hatchery to plan on increasing the mass production of *H. scabra* based on the peak seasons through induce spawning in the captivity.

Acknowledgement
This work was supported by the Ministry of Higher Education under Grant Niche Research Grant Scheme (NRGS) 0002.

References

Agudo, N. (2006). *Sandfish Hatchery Techniques*. Australia: Australian Centre for International Agricultural Research (ACIAR).

Asha, P. S. (2007). Reproductive Biology of the Commercial Sea Cucumber *Holothuria spinifera* (Echinodermata: Holothuroidea) from Tuticorin, Tamil Nadu, India. *Aquaculture International*, (16), 231-242. https://link.springer.com/article/10.1007%2Fs10499-007-9140-z

Benitez-Villalobos, F., Avilla-Poveda, O. H., & Gutierrez-Mendez, I. S. (2013). Reproductive biology of *Holothuria fuscocinerea* (Echinodermata: Holothuroidea) from Oaxaca, Mexico. *Sexuality and Early Development in Aquatic Organisms,* (1), 13-24. http://www.int-res.com/abstracts/sedao/v1/n1/p13-24

Chao, S. M., Chen, C. P., & Alexander, P. S. (1995). Reproductive Cycles of Tropical Sea Cucumbers (Echinodermata: Holothuroidea) in Southern Taiwan. *Marine Biology*, (122), 289-295. https://link.springer.com/article/10.1007/BF00348942

Choo, P.S. (2008). Population Status,Fisheries and Trade of Sea Cucumbers in Asia. In V. Toral-Granda, A. Lovatelli and M. Vasconcellos (Eds). Sea cucumbers. A Global Review of Fisheries and Trade. *FAO Fisheries and Aquaculture Technical Paper*. No. 516 Pp. (81-118). Rome: FAO.

Conand, C. (1993). Reproductive Biology of the Holothurians from the Major Communitiees of the New Caledonia Lagoon. *Marine Biology*, 116: 439-450. https://link.springer.com/article/10.1007/BF00350061

Demeuldre, M., & Eeckhaut, I. (2012). Gonad Development in the Sea Cucumber *Holothuria scabra* Jaeger, 1833. *SPC Beche-de-mer Information Bulletin,* 32: 15-23. http://agris.fao.org/agris-search/search.do?recordID=AV2012082649

Dereli, H., Culha, S. T., Culha, M., Ozalp, B. H., & Tekinay, A.A. (2016). Reproduction and Population Structure of the Sea Cucumber *Holothuria tubulosa* in the Dardanelles Stratit, Turkey. *Mediterranean marine Science,* 47-55.

Dissanayake, D. C. T., & Stefansson, G. (2010). Reproductive biology of the Commercial Sea Cucumber *Holothuria atra* (Holothuroidea: Aspidochirotida) in the Northwestern Coastal Waters of Sri Lanka. *Invertebrate Reproduction and Development, 54* (2), 65-76

Drumm, D. J., & Loneragan, N. R. (2010). Reproductive Biology of *Holothuria lecospilota* in the Cook Islands and the Implications of Traditional Fishing of Gonads on the Population. *New Zealand Journal of Marine and freshwater Research, 39*, 141-156.

Gabr, H. R., Ahmed, A. I., Hanafy, M. H., Lawrence, A. J., Ahmed, M. I., & El-Etreby, S. G. (2004). *Mariculture of Sea Cucumber in the Red Sea- the Egyptian Experience*. Rome: Food and Agriculture Organization of the United Nations.

Guzman, H. M., Guevara, C. A., & Hernandez, I. C. (2003). Reproductive Cycle of Two Commercial Species of Sea Cucumber (Echinodermata: Holothuroidea) from Caribbean Panama. *Marine Biology, 142*: 271-279.

Hasan, M. H. (2005). Destruction of a *Holothuria scabra* Population by Overfishing at Abu Rhamada Island in the Red Sea. *Marine Environmental Research, 60*: 489-511.

Hamel, J-F., & Mercier A. (1996). Studies on The Reproductibe Biology of the Atlantic Sea Cucumber *Cucumaria frondosa*. *SPC Beche-de-mer Information Bulletin*, (8), 22-33.

Hamel, J-F., Conand, C., Pawson, D. L., & Mercier, A. (2001). The Sea cucumber *Holothuria scabra* (Holothuroidea: Echinodermata): Its Biology and Exploitation as Beche-de-Mer. In A. J. Southward., C. M. Young., P. A. Tyler., & L. A. Fuiman (Eds.), *Advance in Marine Biology Volume 41* (129-132). United Kingdom: Academic Press.

Hoareau, T., & Conand, C. (2001). Sexual reproduction of *Stichopus chloronotus*, a Fissiparous Sea Cucumber, on Reunion Island, Indian Ocean. *SPC Beche-de-mer Information Bulletin*, (15): 4-12.

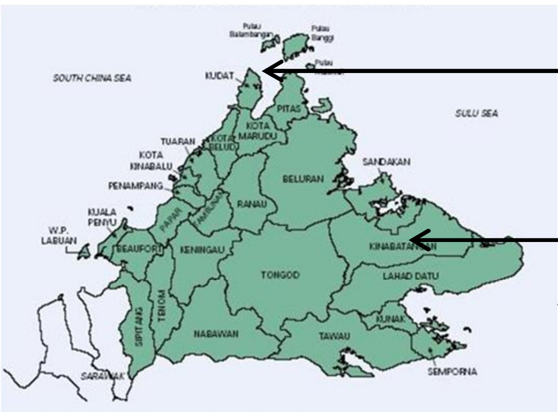
Hopper, D. R., Hunter, C. L., & Richmond, R. H. (1998). Sexual Reproduction of the Troical Sea Cucumber, *Actinopyga mauritiana* (Echnodermata; Holothuroidea), in Guam. *Bulletin of Marine Science, 63* (1), 1-9

James, D. B. (1989). *Marine Fisheries Information Service Special Issue on Beche-de-mer*. India: Central Marine Fisheries Research Institute Cochin, India.

James, P., Zhou, S., & Prasetyo, A. P. (2015). Soft Bodies Make Estimation Hard; Correlations Among Body Dimensions and Weights of Multiple Species of Sea Cucumbers. *Marine and Freshwater Research,* 66, 857-865.

Kamarudin, K. R., Rehan, A. M. Hashim,R., & Usup, G. (2010). An Update on Diversity of Sea Cucumbers (Echinodermata: Holothuroidea) in Malaysia. *Malayan nature Journal, 62* (3): 315-334.

Keshavarz, M., Mohammadikia, D., Dabbagh, A. R., & Kamrani, E. (2015). Reproductive Biology of the Sea Cucumbers for Successful Breeding: a Review. *Journal of Animal Production Advances, 2* (5):208-213.
Kithakeni, T., & Ndaro, S. G. M. (2002). Some Aspects of Sea Cucumber, *Holothuria scabra* (Jaeger,1935), along the Coast of Dar es Salaam. *Western Indian Ocean J. Mar. Sci, 1* (2):163-168.
Kuganathan, S. (2014). *Sea Cucumbers Status and Culture Potential in the Jaffna Lagoon, Sri Lanka*. Sri Lanka: Department of Fisheries University of Jaffna, Sri Lanka.
Mercier, A., Battaglene, S. C., & Hamel, J-F. (2000). Periodic Movement, Recruitment and Size-Related Distribution of the Sea Cucumber *Holothuria scabra* in Solomon Islands. *Hydrobiologia*, 440; 81-100.
Muthiga, N. A., & Kawaka, J. (2008). The Effects of Temperature and Light on the Gametogenesis and Spawning of Four Sea Urchin and One Sea Cucumber Species on Coral Reefs in Kenya.
Omar H. A., Abdel Razek, F. A., Abdel Rahman, S. H., & El Shimy, N. A. (2013). Reproductive Periodicity of Sea Cucumber *Bohadschia vitiensis* (Echinodermata: Holothuroidea) in Hurghada Area, Red Sea, Egypt. *Egyption Journal of Aquatic Research,* (39), 115-123.
Pangkey, H., Lantu, S., Manuand, L., & Mokolensang, J. (2012). Prospect of Sea Cucumber Culture in Indonesia as Potential Food Sources. *Journal of Coastal Development, 15* (2):114-124.
Pitt, R., & Duy, N. D. Q. (2003). Breeding and Culture of the Sea Cucumber *Holothuria scabra* in Vietnam. *Aquaculture Asia,8* (1): 36-39.
Plotieau, T., Baele, J-M., Vaucher, R., Hasler, C-A., Koudad, D., & Eeckhaut, I. (2013). Analysis of the Impact of *Holothuria scabra* Intensive Farming on Sediment. *Cah. Biol. Mar, 54*: 703-711.
Preston, G. (1993) Chapter 11: Bêche-de-mer. In: A. Wright & L. Hill (Eds.), *Inshore Marine Resources of the South Pacific: Information for Fishery Development and Management* (371-407). Fiji: FFA/USP Press.
Purcell, S. W., Lovatelli, A., Vasconcellos, M., & Ye, Y. (2010). *Managing Sea Cucumber Fisheries with an Ecosystem Approach*. Rome, Italy. Food and Agriculture Organization of the United Nations.
Purcell, S. W., Samyn, Y., & Conand, C. (2012). *Commercially Important Sea Cucumbers of the World.* Rome: Food and Agriculture Organization of the United Nations.
Purwati, P. (2006). Reproductive Pattern of Holothuria scabra (Echinodermata: Holothuroidea) in Indonesian Waters. *Mar. Res. Indonesia,* (30), 47-55.
Rahman, M. A. (2014). Global Sea Cucumber Fisheries: Their Culture Potentials, Boactive Compounds and Sustainable Utilizations. International Journal of Advances in Chemical Engg., *Biological Sciences, 1*(2): 193-197.
Sicuro, B., & Levine, J. (2011). Sea Cucumber in the Mediterranean: A Potential Species for Aquaculture in the Mediterranean. *Reviews in Fisheries Science, 19* (3): 299-304.
Ramofafia, C., Byrne, M., & Battaglene, C. S. (2003). Reproduction of the Commercial Sea Cucumber *Holothuria scabra* (Echinodermata: Holothuroidea) in the Solomon Islands. *Marine Biology, 142*: 281-288.
Rasolofonirina, R., Vaitilingon, D., Eeckhaut, I., & Jangoux, M. (2005). Reproductive Cycle of Edible Echinoderms from the South-Western Indian Ocean. *Western Indian Ocean J. Mar. Sci. 4* (1): 61-75.
Reichenbach, N. (1999). Ecology and fishery Biology of *Holothuria fuscogilva* in the Maldives Indian Ocean (Echinodermata: Holothuroidea). *Bulletin of Marine Science, 64* (1), 103-113



Limau-Limauan, Kudat
N06 49'24.4",
E116 51'42.0"

Telaga Tujuh, Kunak
N04 39'52.05",
E118 15'49.01"

ak, Sabah

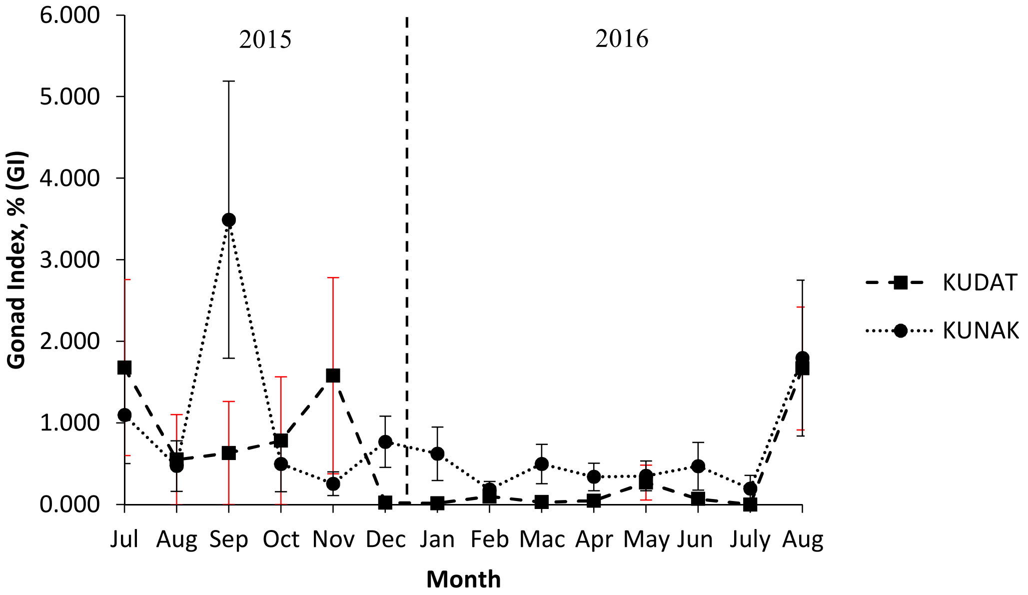
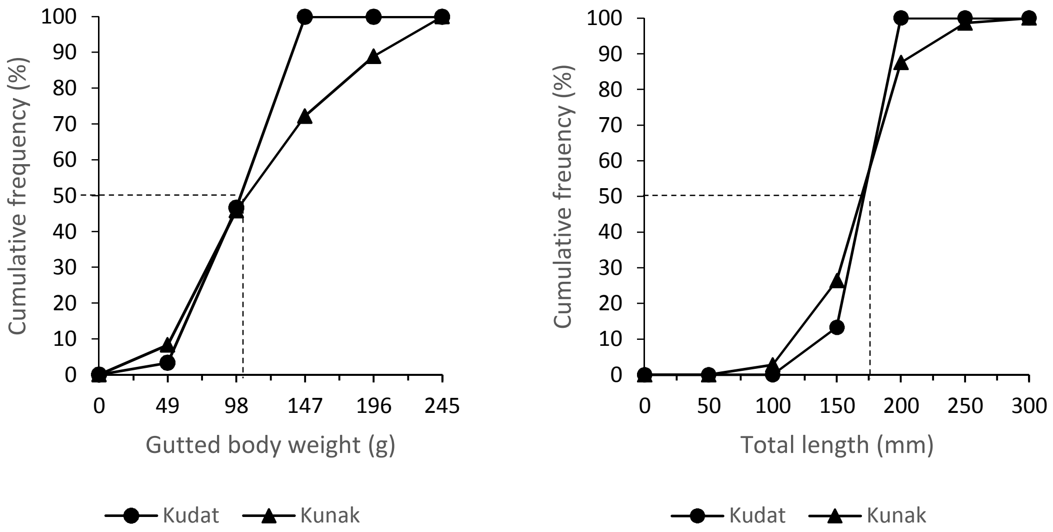


Figure 2- Gonad index (%) distribution at Kudat and Kunak from July 2015 to August 2016



a) b)

Figure 3- Size at first sexual maturity based on; a) gutted body weight (GBW_{50}) and b) total length (TL_{50}) at Kudat and Kunak

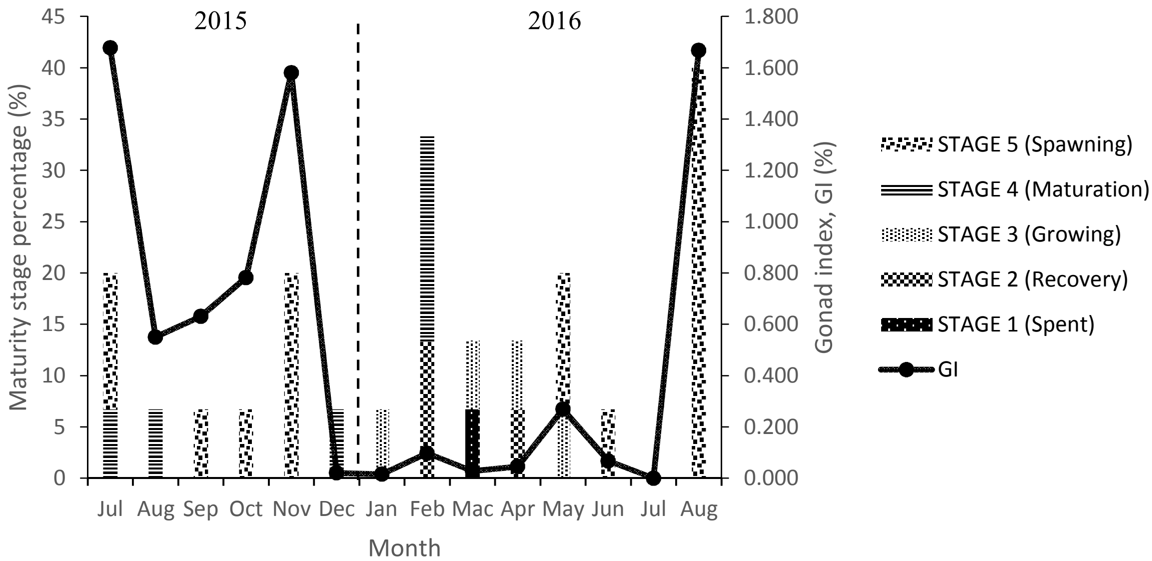


Figure 4- Maturity stage percentage and GI from July 2015 to August 2016 in Kudat

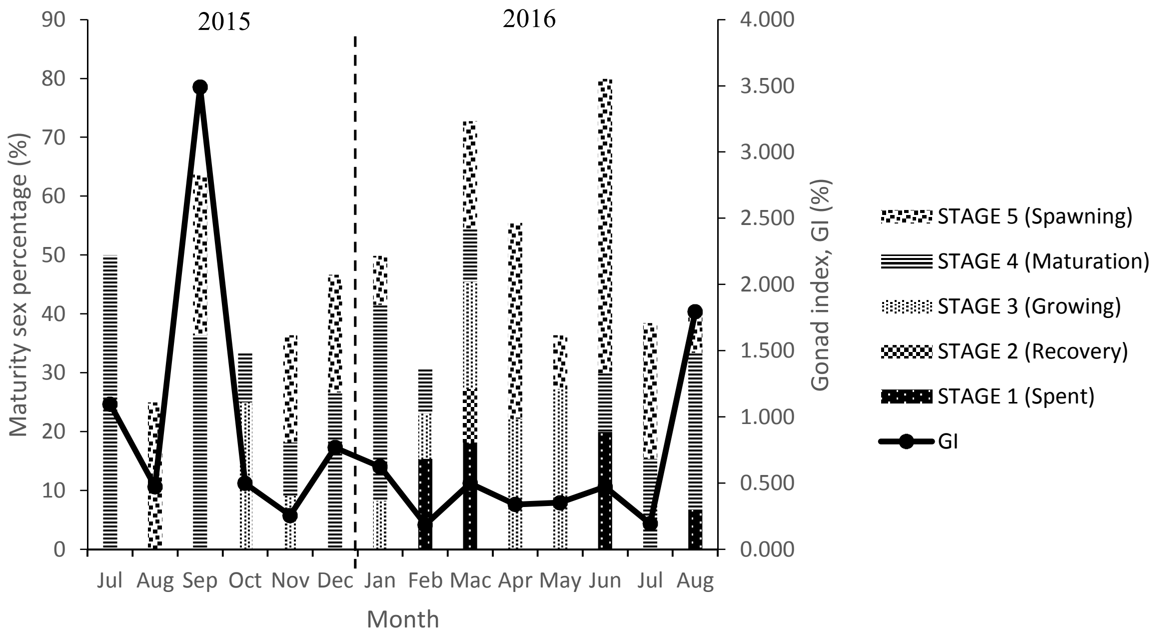
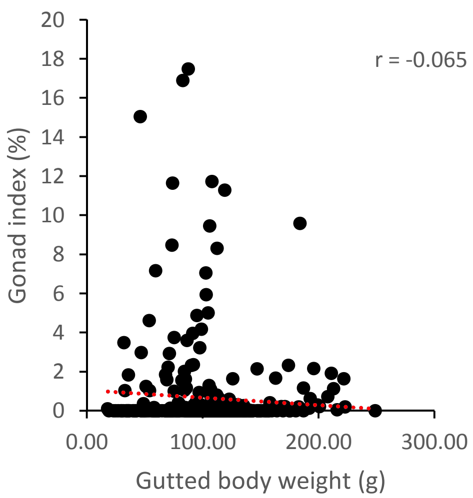
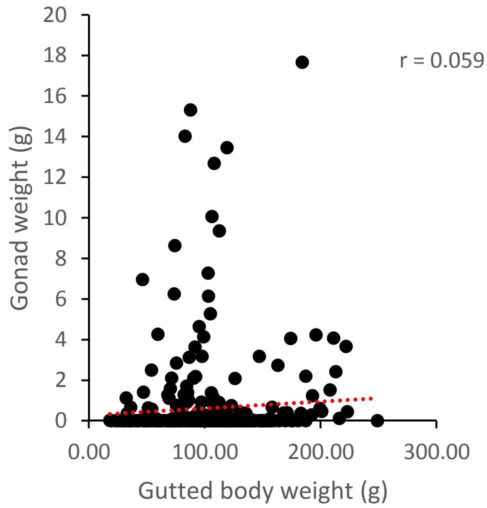


Figure 5- Maturity stage percentage and GI from July 2015 to August 2016 in Kunak





a) b)

Figure 6- Relationship between Gutted body weight (g) with (a) gonad index (%), and (b) gonad weight (g). (n= 369, p> 0.01)

Table 1- Microscopic description of male and female gonad of *H. scabra*. (Ramolofonirina *et al.,* 2005).

Stage	Male	Female
I (Spent)	- Tubules are translucent to whitish - No parietal germinal cells seen but a few scattered	- Tubules are translucent to whitish - Tubule lumen may fill with relict oocytes with a few somatic cells. - No parietal germinal cells seen but a few scattered oogonia
II (Recovery)	- Thin layer of germinal cell of male tubules - Recovery tubules are translucent - Tubule wall is rather thick and lumen usually empty	- Tubules may include small previtellogenetic oocytes in diameter - Recovery tubules are translucent - Tubule wall is rather thick and lumen usually empty
III (Growing)	- Tubules have white colour - Ridges of connective tissue develop towards the centre of the lumen and spermatogonia and spermatocytes are present. - An empty space still visible in the centre where a few spermatozoa can be observed	- Tubules have yellow colour - Oocytes start to grow (from 20µm to 120µm)
IV (Maturation)	- Tubules are whitish to yellow cream - Lumen of male tubule is filled with spermatozoa and spermatocytes	- Tubules are clear to dark orange - A few previtellogenic oocytes and most of large vitellogenic oocytes fill almost completely tubule lumen
V (Spawning)	- Tubules are pale yellow - Spermatozoa fill most of the tubule lumen	- Tubules are orange - Ovarian tubules are filled with large, rounded oocytes.

Table 2- Size distribution of *H. scabra* in Kudat and Kunak (Mean ± S.E).

Place	Total length (mm)	Total body weight (g)	Gutted body weight (g)	Gonad weight (g)
Kudat	174.85 ± 1.27[a]	242.11 ± 3.61[a]	110.84 ± 1.58[a]	0.47 ± 0.13[a]
Kunak	162.66 ± 2.68[b]	203.99 ± 7.67[b]	97.96 ± 3.97[b]	0.84 ± 0.18[a]

*Different superscript at same column indicates significant different (p<0.05).

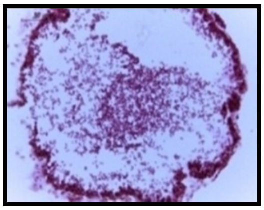
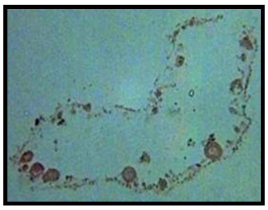
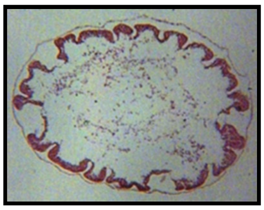
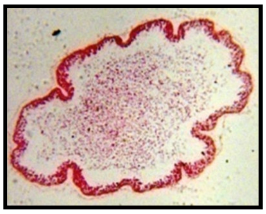
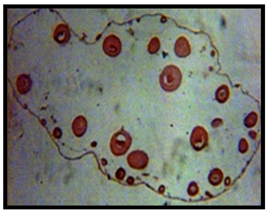
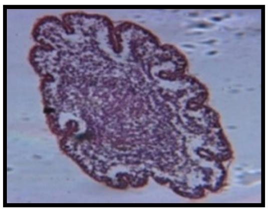
Table 3: Percentage of *H. scabra* sex in Kudat from July 2015 to August 2016

Year	Month	n	Male (%)	Female (%)	Unsexed (%)
2015	July	15	20	0	80
	August	15	0	6.7	93.3
	September	15	6.7	0	93.3
	October	15	0	6.7	93.3
	November	15	6.7	13.3	80
	December	15	0	6.7	93.3
2016	January	15	6.7	0	93.3
	February	15	20	13.3	66.7
	March	15	6.7	6.7	86.6
	April	15	0	13.3	86.7
	May	15	13.3	6.7	80
	June	15	6.7	0	93.3
	July	15	0	0	100
	August	15	13.3	26.7	60

Table 4: Percentage of *H. scabra* sex in Kunak from July 2015 to August 2016

Year	Month	n	Male (%)	Female (%)	Unsexed (%)
2015	July	8	12.5	37.5	50
	August	8	0.0	25.0	75
	September	11	45.5	18.2	36.3
	October	12	16.7	8.3	75
	November	11	9.1	27.3	63.6
	December	15	20.0	26.7	53.3
2016	January	12	33.3	8.3	58.4
	February	13	23.1	7.7	69.2
	March	11	54.5	18.2	27.3
	April	9	44.4	11.1	44.5
	May	11	9.1	27.3	63.6
	June	10	60.0	20.0	20.0
	July	13	30.8	7.7	61.5
	August	15	20.0	20.0	60.0

Table 5- Histological examinations of male and female gonad of *H. scabra*

Stage	**Male**	**Female**
I (Spent)		
II (Recovery)		
III (Growing)		
IV (Maturation)		
V (Spawning)